SEQUENCE LISTING

110> EIJSINK, VINCENT et al. EXPRESSION SYSTEM IN MICROORGANISM AND ITS USE FOR EXPRESSING MAY 3 0 2003 HETEROLOGOUS AND HOMOLOGOUS PROTEINS 1380-0122P <140> US 09/068,507 <141> 1998-07-15 <160> 19 <170> PatentIn version 3.1 <210> 1 <211> 26 <212> PRT <213> Lactobacillus plantarum <400> 1 Lys Ser Ser Ala Tyr Ser Leu Gln Met Gly Ala Thr Ala Ile Lys Gln Val Lys Lys Leu Phe Lys Lys Trp Gly Trp <210> 2 <211> 114 <212> DNA <213> Lactobacillus sake <220> <221> CDS <222> (1)..(114) <223> <400> 2 48 atg atg ata ttt aaa aaa ctt tca gaa aaa gaa ttg caa aaa ata aac Met Met Ile Phe Lys Lys Leu Ser Glu Lys Glu Leu Gln Lys Ile Asn 96 ggt ggt atg gca gga aat agt tct aat ttt att cat aag att aaa caa Gly Gly Met Ala Gly Asn Ser Ser Asn Phe Ile His Lys Ile Lys Gln 25 20 114 att ttt acc cat cgt taa Ile Phe Thr His Arg 35 <210> 3

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Ile Leu Leu Ile Phe Leu Leu Tyr Arg Tyr Ile Gln Arg Lys Ile Thr	·
Ile Leu Leu Ile Phe Leu Leu Tyr Arg Tyr Ile Gln Arg Lys Ile Thr 20 25 30 Phe Lys Arg Ile Ile Leu Asp Ile Leu Ile Ala Ile Ile Phe Ser Ile	
Ile Leu Leu Ile Phe Leu Leu Tyr Arg Tyr Ile Gln Arg Lys Ile Thr 20 Phe Lys Arg Ile Ile Leu Asp Ile Leu Ile Ala Ile Ile Phe Ser Ile 40 Leu Tyr Leu Phe Ile Ser Asp Ala Ser Leu Leu Val Met Val Leu Met	
Ile Leu Leu Ile Phe Leu Leu Tyr Arg Tyr Ile Gln Arg Lys Ile Thr 20 Phe Lys Arg Ile Ile Leu Asp Ile Leu Ile Ala Ile Ile Phe Ser Ile 40 Leu Tyr Leu Phe Ile Ser Asp Ala Ser Leu Leu Val Met Val Leu Met 50 Arg Leu Gly Trp His Phe His Gln Gln Lys Glu Asn Lys Ile Lys Thr	

Ser	Asp	Phe 115	Ser	Gln	Asn	Ile	Leu 120	Asn	Asn	Ser	Ala	Thr 125	Asp	Ile	Thr
Leu	Leu 130	Gly	Ile	Phe	Phe	Ala 135	Val	Leu	Phe	Asp	Gly 140	Leu	Phe	Phe	Ile
Leu 145	Leu	Lys	Asn	Lys	Arg 150	Thr	Glu	Leu	Gln	His 155	Leu	Asn	Gln	Glu	Ile 160
Ile	Glu	Phe	Ser	Leu 165	Glu	Lys	Gln	Tyr	Phe 170	Ile	Phe	Ile	Phe	Ile 175	Leu
Phe	Ile	Val	Ile 180	Glu	Ile	Ile	Leu	Ala 185	Val	Gly	Asn	Leu	Gln 190	Gly	Val
Thr	Ala	Thr 195	Ile	Leu	Leu	Thr	Ile 200	Ile	Ile	Ile	Phe	Cys 205	Val	Leu	Ile
Gly	Met 210	Thr	Phe	Trp	Gln	Val 215	Met	Leu	Phe	Leu	Lys 220	Ala	Tyr	Ser	Ile
Arg 225	Gln	Glu	Ala	Asn	Asp 230	Gln	Leu	Val	Arg	Asn 235	Gln	Gln	Leu	Gln	Asp 240
Tyr	Leu	Val	Asn	Ile 245	Glu	Gln	Gln	Tyr	Thr 250	Glu	Leu	Arg	Arg	Phe 255	Lys
His	Asp	Tyr	Gln 260	Asn	Ile	Leu	Leu	Ser 265	Leu	Glu	Ser	Phe	Ala 270	Glu	Lys
Gly	Asp	Gln 275	Gln	Gln	Phe	Lys	Ala 280	Tyr	Tyr	Gln	Glu	Leu 285	Leu	Ala	Gln
Arg	Pro 290	Ile	Gln	Ser	Glu	Ile 295	Gln	Gly	Ala	Val	Ile 300	Ala	Gln	Leu	Asp
Tyr 305	Leu	Lys	Asn	Asp	Pro 310	Ile	Arg	Gly	Leu	Val 315	Ile	Gln	Lys	Phe	Leu 320
Ala	Ala	Lys	Gln	Ala 325	Gly	Val	Thr	Leu	Lys 330	Phe	Glu	Met	Thr	Glu 335	Pro

Ile Glu Leu Ala Thr Ala Asn Leu Leu Thr Val Ile Arg Ile Ile Gly
340 345 350

Ile Leu Leu Asp Asn Ala Ile Glu Gln Ala Val Gln Glu Thr Asp Gln 355 360 365

Leu Val Ser Cys Ala Phe Leu Gln Ser Asp Gly Leu Ile Glu Ile Thr 370 375 380

Ile Glu Asn Thr Ala Ser Gln Val Lys Asn Leu Gln Ala Phe Ser Glu 385 390 395 400

Leu Gly Tyr Ser Thr Lys Gly Ala Gly Arg Gly Thr Gly Leu Ala Asn 405 410 415

Val Gln Asp Leu Ile Ala Lys Gln Thr Asn Leu Phe Leu Glu Thr Gln \$420\$ \$430\$

Ile Glu Asn Arg Lys Leu Arg Gln Thr Leu Met Ile Thr Glu Glu Thr 435 440 445

<210> 15

<211> 248

<212> PRT

<213> Lactobacillus sakei

<400> 15

Leu Tyr Pro Val Tyr Leu Leu Glu Asp Asp Leu Gln Gln Gln Ala Ile 1 5 10 15

Tyr Gln Gln Ile Ile Ala Asn Thr Ile Met Ile Asn Glu Phe Ala Met 20 25 30

Thr Leu Thr Cys Ala Ala Ser Asp Thr Glu Thr Leu Leu Ala Ala Ile 35 40 45

Lys Asp Gln Gln Arg Gly Leu Phe Phe Leu Asp Met Glu Ile Glu Asp 50 55 60

Asn Arg Gln Ala Gly Leu Glu Val Ala Thr Lys Ile Arg Gln Met Met 65 70 75 80

Pro Phe Ala Gln Ile Val Phe Ile Thr Thr His Glu Glu Leu Thr Leu

85 90 95

Leu	Thr	Leu	Glu	Arg	Lys	Ile	Ala	Pro	Leu	Asp	Tyr	Ile	Leu	Lys	Asp	
			100					105					110			

Gln Thr Met Ala Glu Ile Lys Arg Gln Leu Ile Asp Asp Leu Leu Leu 115 120 125

Ala Glu Lys Gln Asn Glu Ala Ala Ala Tyr His Arg Glu Asn Leu Phe 130 135 140

Ser Tyr Lys Ile Gly Pro Arg Phe Phe Ser Leu Pro Leu Lys Glu Val 145 150 155 160

Val Tyr Leu Tyr Thr Glu Lys Glu Asn Pro Gly His Ile Asn Leu Leu 165 170 175

Ala Val Thr Arg Lys Val Thr Phe Pro Gly Asn Leu Asn Ala Leu Glu 180 185 190

Ala Gln Tyr Pro Met Leu Phe Arg Cys Asp Lys Ser Tyr Leu Val Asn 195 200 205

Leu Ser Asn Ile Ala Asn Tyr Asp Ser Lys Thr Arg Ser Leu Lys Phe 210 215 220

Val Asp Gly Ser Glu Ala Lys Val Ser Phe Arg Lys Ser Arg Glu Leu 225 230 235 240

Val Ala Lys Leu Lys Gln Met Met 245

<210> 16

<211> 61

<212> PRT

<213> Lactobacillus sakei

<400> 16

Met Glu Lys Phe Ile Glu Leu Ser Leu Lys Glu Val Thr Ala Ile Thr 1 5 10 15

Gly Gly Lys Tyr Tyr Gly Asn Gly Val His Cys Gly Lys His Ser Cys 20 25 30

Thr Val Asp Trp Gly Thr Ala Ile Gly Asn Ile Gly Asn Asn Ala Ala 35 40 45

Ala Asn Trp Ala Thr Gly Gly Asn Ala Gly Trp Asn Lys 50 55 60

<210> 17

<211> 98

<212> PRT

<213> Lactobacillus sakei

<400> 17

Met Lys Ile Leu Lys Trp Tyr Ser Gly Gly Lys Asp Arg Gly Glu Arg
1 5 10 15

Ala Asn Asp Ile Ile Gly Gln Leu Leu Leu Asp Leu Asn His Asp Pro 20 25 30

Lys Asn Glu His Leu Glu Ala Ile Leu Ile Asn Tyr Gln Asn Glu Ile $35 \hspace{1cm} 40 \hspace{1cm} 45$

Lys Arg Lys Glu Ser Ser Val Pro Phe Ile Leu Ser Arg Met Asn Ile 50 55 60

Ser Ile Ala Asn Thr Ile Arg Arg Asp Arg Leu Ile Leu Thr Asp Phe 65 70 75 80

Gln Glu Asp Lys Leu Lys Leu Leu Thr Ala Leu Ser Asn Ile Arg Tyr 85 90 95

Gly Tyr

<210> 18

<211> 718

<212> PRT

<213> Lactobacillus sakei

<400> 18

Met Ile Asp Arg Lys Ile Tyr Ile Pro Gln Val Asp Glu Met Asp Cys 1 5 10 15

Gly Val Ala Ala Leu Ala Met Ile Leu Lys His Tyr Gly Ser Ser Ala 20 25 30

Ser Leu Ala Tyr Leu Arg Asn Glu Ala Lys Thr Asp Leu Glu Gly Thr Thr Ala Leu Gly Leu Val Lys Thr Ala Glu Asn Leu Gly Phe Glu Thr Lys Ala Ile Gln Ala Asp Met Ser Leu Phe Glu Val Ala Asp Leu Pro Phe Pro Phe Ile Ala His Val Leu Lys Asn Gly Glu Leu Leu His Tyr Tyr Val Val Leu Gly Ile Lys Lys Asp Ala Ile Ile Ile Ala Asp Pro Asp Pro Thr Val Gly Ile Ala Lys Met Thr Gln Ser Gln Phe Lys Ser Glu Trp Thr Gly Val Ala Leu Phe Ile Ala Pro Lys Pro Ser Tyr Gln Pro Val Lys Gln Gln Thr Lys Ala Ser Leu Phe Ser Phe Val Pro Thr Leu Met Gln Gln Lys Arg Leu Val Ile Asn Ile Thr Leu Ala Ala Leu Leu Ile Thr Ile Ile Ser Ile Leu Gly Ser Tyr Phe Leu Gln Thr Val Ile Asp Thr Tyr Ile Pro Asn Asn Met Gln Ser Thr Leu Ala Ile Val . Ala Ile Gly Leu Ile Ile Phe Tyr Gly Phe Gln Ser Ile Phe Thr Tyr Ala Gln Asn Phe Leu Leu Ala Val Leu Gly Gln Arg Leu Ser Ile Glu

Ile Ile Leu Ser Tyr Leu Arg His Val Phe Glu Leu Pro Met Ser Phe

Phe Ala Thr	Arg Lys 260	Thr Gly		Ile 265	Ile	Ser	Arg	Phe	Asn 270	Asp	Ala
Asn Lys Ile 275	Ile Asp	Ala Leu	Ala 280	Ser	Ala	Ile	Ile	Ser 285	Ile	Phe	Leu
Asp Ile Thr 290	Val Val	Val Ile 295		Gly	Ile	Ile	Leu 300	Ile	Ile	Gln	Asn
Thr Thr Leu 305	Phe Trp	Leu Thr 310	Leu	Ala	Val	Ile 315	Pro	Ile	Tyr	Ile	Ala 320
Val Ile Thr	Leu Phe 325	Ala Lys	Pro	Phe	Glu 330	Lys	Leu	Asn	Gln	Lys 335	Glu
Met Glu Ser	Asn Ala 340	Val Leu	Asn	Ser 345	Ala	Ile	Ile	Glu	Asp 350	Leu	His
Gly Ile Glu 355	Thr Ile	Lys Ala	Leu 360	Thr	Ser	Glu	Ser	Glu 365	Arg	Tyr	Gln
Lys Ile Asp 370	Thr Ala	Phe Val 375	_	Tyr	Leu	Lys	Lys 380	Ser	Phe	Asp	Tyr
Val Lys Ala 385	Asp Thr	Leu Gln 390	Gln	Ala	Ile	Lys 395	Leu	Phe	Val	Gln	Leu 400
Gly Leu Asn	Val Phe 405	Val Leu	Trp	Val	Gly 410	Ala	Ile	Leu	Val	Thr 415	His
Asn Glu Leu	Ser Ile 420	Gly Gln	Leu	Met 425	Thr	Tyr	Asn	Ala	Leu 430	Leu	Ala
Tyr Phe Val 435	Asn Pro	Leu Gln	Asn 440	Ile	Ile	Asn	Leu	Gln 445	Thr	Lys	Leu
Gln Ser Ala 450	Arg Val	Ala Asn 455	Asn	Arg	Leu	Asn	Glu 460	Val	Tyr	Leu	Val
Asp Ser Glu 465	Phe Lys	Thr Glu 470	Arg	Pro	Ile	Lys 475	Ser	Glu	Ser	Gln	Leu 480

Val Gly Pro Ile Glu Leu Gln Asn Val Ser Tyr Gln Tyr Gly Tyr Gly Gln Arg Val Leu Glu Ser Ile Ser Leu Lys Ile Glu Pro Asn Glu Lys Leu Thr Ile Val Gly Met Ser Gly Ser Gly Lys Ser Thr Leu Val Lys Leu Leu Val Asn Phe Phe Glu Pro Thr Glu Gly Lys Val Leu Phe Asn Gln Gln Asp Thr Gln Thr Ile Asp Lys Lys Thr Leu Arg Gln Phe Val Asn Tyr Ile Ala Gln Thr Pro Tyr Ile Phe Ser Gly Ser Ile Trp Asp Asn Leu Lys Leu Gly Asn Arg Pro Gly Ile Thr Gln Ala Asp Ile Met His Ala Cys Glu Leu Ala Glu Ile Lys Asn Asp Ile Glu Lys Met Pro Leu Gln Phe Glu Thr Leu Leu Asp Glu Asp Gly Asn Thr Leu Ser Gly Gly Gln Lys Gln Arg Leu Thr Ile Ala Arg Ala Leu Leu Ser Pro Ala Arg Val Leu Ile Phe Asp Glu Ser Thr Ser Gly Leu Asp Thr Ile Thr Glu Gly Leu Leu Val Asp His Leu Met Ala Leu Glu Glu Arg Thr Ile Ile Phe Ile Ala His Arg Leu Ala Ile Ala Lys Lys Thr Asn Asn Ile

Leu Val Asp Thr Gln Gly Arg Tyr Tyr Gln Leu Leu Asn His

Leu Val Leu Glu Asn Gly Ser Val Val Glu Thr Gly Thr His Asp Ala

705 710 715

<210> 19 <211> 458 <212> PRT <213> Lactobacillus sakei

<400> 19

Met Asp Lys Glu Phe Leu Glu Ser Ser Glu Phe Tyr Ser Ala Arg Phe 1 5 10 15

Lys Asn Phe Ser Thr Leu Leu Ile Met Pro Ile Ala Val Leu Leu Cys 20 25 30

Leu Val Cys Ile Phe Ser Phe Phe Gly Lys Arg Glu Ile Thr Ile Glu 35 40 45

Gly Gln Gly Asp Leu Thr Thr Asn Lys Gln Ile Pro Ile Leu Gln Ala 50 55 60

Ser Thr Asn Ser Val Leu Lys Gln Asn Tyr Leu Lys Glu Gly Lys Phe 70 75 80

Val Lys Lys Gly Gln Thr Leu Leu Val Tyr Gln Asn Thr Lys Asn Gln 85 90 95

Asn Gln Lys Arg Leu Leu Glu Lys Gln Gln Asp Asn Leu Ser Tyr Gln
100 105 110

Ile Thr Ser Leu Glu Thr Leu Lys Ala Ser Ile Arg Ser Asn Gln Asp 115 120 125

.Gln Phe Glu Asn Asn Asp Gln Phe Gly Tyr Arg Asp Leu Leu Arg Gly 130 135 140

Tyr Leu Asp Gln Arg Gln Val Tyr Leu Ile Glu Asn Gln Met Val Thr 145 150 155 160

Asp Lys Ala Ala Thr Ser Thr Thr Lys Gln Gln Thr Leu Thr Ala Ile 165 170 175

Met Asp Lys Thr Ile Gln Arg Asp Gln Thr Asn Leu Asp Ala Tyr Gln 180 185 190

Ala	Leu	Tyr 195	Gln	Ser	Ile	Lys	Thr 200	Asn	Lys	Val	Tyr	Ser 205	Ser	Thr	Gly
Lys	Tyr 210	Asn	Tyr	Leu	Tyr	Glu 215	Asn	Tyr	Gln	Thr	Lys 220	Val	Lys	Ala	Met
Ser 225	Asp	Lys	Asn	Glu	Gln 230	Ser	Gly	Leu	Lys	Ala 235	Asp	Ile	Leu	Ala	Thr 240
Thr	Gln	Gln	Gln	Ile 245	Asp	Ser	Leu	Gln	Gly 250	Ser	Ile	Ala	Asn	Ala 255	Glu
Asn	Gln	Lys	Val 260	Ser	Ile	Lys	Glu	Phe 265	Asp	Asp	Ser	Arg	Phe 270	Asn	Thr
Asn	Thr	Asn 275	Asn	Glu	Lys	Leu	Ala 280	Leu	Leu	Glu	Ala	Gln 285	Gln	Thr	Gln
Ser	Ala 290	Asn	Glu	Gln	Leu	Val 295	Lys	Ala	Lys	Gln	Ser 300	Leu	Val	Glu	Ile
Gln 305	Thr	Thr	Leu	Lys	Gln 310	Ile	Thr	Ser	Asp	Ser 315	Lys	Glu	Tyr	Val	Val 320
Lys	Ala	Pro	Lys	Asp 325	Gly	Val	Leu	His	Val 330	Asp	Asp	His	Tyr	Gln 335	Gly
Ile	Lys	Tyr	Thr 340	Ser	Ala	Gly	Thr	Ser 345	Met	Ala	Arg	Ile	Tyr 350	Pro	Val
Leu	Ala	Asp .355	Gln	Lys	Arg	Leu	Lys 360	Ile	Glu	Ala	Leu	Ile 365	Pro	Val	Asp
Asp	Ile 370	Ser	Ser	Val	Lys	11e 375	Gly	Gln	Arg	Leu	Arg 380	Leu	Lys	Ile	Thr
Arg 385	Asn	Val	Pro	Arg	Pro 390	Ile	Ile	Ile	Glu	Gly 395	Lys	Ile	Asn	Gln	Ile 400
Ser	Val	Ser	Pro	Thr 405	Val	Ile	Asn	Gln	Gly 410	Ser	Tyr	Tyr	Thr	Ile 415	Ile

Gly Leu Ala Thr Ile Ser Asn Asn Asn Arg Lys Leu Leu His Tyr Gly 420 425 430

Met Thr Gly Lys Ile Ala Ile Ile Thr Gly Lys Thr Thr Phe Phe Asn $435 \hspace{1.5cm} 440 \hspace{1.5cm} 445 \hspace{1.5cm}$

Tyr Tyr Lys Asp Lys Leu Leu Asn Arg Asn 450 455